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Methodical Approach for Identifying Changes in Xylem Vulnerability of *Asparagus officinalis* L. During the Vegetation Period

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Summary

This study focuses on the characterization of the xylem vulnerability of *Asparagus officinalis* L. to cavitation by calculating the drought-induced loss of hydraulic conductivity (K) per xylem water potential (ψ_{xyl}). This relationship is used to create vulnerability curves (VC) and to derive a drought stress threshold applicable for irrigation purposes. Three shoot treatments for generating VC and for revealing the drought resistance of this species are compared: the "diurnal transpiration treatment", the "soil drying treatment" and the "lab transpiration treatment". The "lab transpiration treatment", using excised, rehydrated and subsequently dehydrated shoots, was selected as the best method to produce VC compared to natural dehydration treatments. Using this method, VC were established for field-grown plants over the season to detect changes in xylem vulnerability with time. The cavitation resistance decreased during the period, when shoots were fully developed but not senescent. Asparagus experienced 50 % loss of K when ψ_{xyl} at predawn reached −0.3 MPa in July and −0.2 MPa in September. This seasonal shift of the critical ψ_{xyl} has to be taken into consideration for plant-based irrigation scheduling using ψ_{xyl} as irrigation signal.

Key words. Hydraulic conductivity – xylem cavitation – cavitation resistance – vulnerability curve – water flow – primary xylem – native embolism – monocot

Introduction

A high level of hydraulic conductivity (K) in xylem indicates an unlimited water transport from roots to shoots. Drought conditions during crop production result in a detrimental decrease in K by releasing cavitation in the vessels, and cause a decrease of xylem water potential (ψ_{xyl}). Changes of K are closely linked to even very small changes in ψ_{xyl}. Thus, K may hold a key role in feedforward mechanisms triggering drought avoidance by stomatal closure. There is a need to assess K in vegetable crops due to the lack of information on the response of K to changing environmental conditions, particularly soil and air water deficit. The water transport capacity of herbaceous species may alter due to changes in weather conditions, in ecosystems or in growing conditions of agricultural species (Kocacinar and Sage 2003; Maherali et al. 2004). Temporarily modified growth conditions such as water availability alter K in shoots by releasing or refilling cavitation in xylem elements (Zimmermann 1983; Sperry and Tyree 1988; Bucci et al. 2003). The degree of these effects on K is to some extent genetically determined, indicating a genotype-specific cavitation resistance to drought stress (Kavanagh et al. 1999; Kolb and Sperry 1999). The determination of cavitation resistance is enabled by relating changes of K to ψ_{xyl} and, in this way, by establishing xylem vulnerability curves (VC). Measuring ψ_{xyl} may potentially be used to aid irrigation scheduling, as shown for walnut trees (Améglio et al. 1999) and grapevines (Gruber, unpublished). VC help us to understand the functional implications of different values of ψ_{xyl} and could therefore be used to help determining appropriate threshold levels of ψ_{xyl}.

In this study, we used the canopy of *Asparagus officinalis* L. for establishing VC, since it is the vegetable crop with the greatest economic importance and the largest production area in Central-Europe (Behr 2009). For measuring K, several methods have been reported previously. Most of them have been developed for woody species, while the number of studies on herbaceous species (Tsuda and Tyree 2000; Nijsse et al. 2001; Cochard 2002; Kocacinar and Sage 2003; Stiller et al. 2003), as well as those using leaf blades or petioles increased during the last decades (Sperry and Pockman 1993; Zwieniecki et al. 2000; Bucci et al. 2003). Unfortunately, these methods are not suitable for water conducting tissue of monocot herbaceous plant material, such as asparagus, with very long xylem vessels consisting exclusively of primary xylem. Consequently, Schaller and Paschold (2008) adapted the above-mentioned methods and established a technique for measuring K in asparagus shoots. Nevertheless, appropriate methods for lowering ψ_{xyl} in asparagus tissue and inducing losses of K under near-natural conditions have not been reported yet.

This study examines drought-inducing treatments for generating xylem embolism to determine changes in xylem

vulnerability of asparagus during the season. Based on the foregoing methodical approach, the hypothesis is tested, that cavitation resistance of asparagus is subjected to seasonal changes.

Materials and Methods

Plant material and growth conditions

Plant material of *Asparagus officinalis* L. cultivars 'Gijnlim' and 'Grolim' was sourced from well-watered plants in two different years and two cultivation conditions at the Geisenheim Research Center in Germany (49° 59' 5" N 7° 56' 55" E). Soil suction was held above −200 hPa via drip irrigation. In 2006, one-year-old plants of 'Gijnlim' and 'Grolim' in 15 l pots filled with loamy sand were cultivated in a greenhouse in order to reveal coherences under controlled conditions. In 2008, ten-year-old plants of 'Gijnlim' grown in the field on sandy loam were used for validating VC under field conditions. In 2006 and 2008 experiments started when shoots were fully differentiated – at the beginning of July – and ended at mid-September before senescence. According to the segmentation hypothesis (ZIMMERMANN 1983), K was investigated in lateral shoots, since these are more susceptible to embolism than the main stem (SPERRY and POCKMAN 1993; MAYR et al. 2003).

Shoot treatments and xylem water potential measurement

Three shoot dehydration treatments were applied to induce changes in water potential (ψ) of potted plants. In the first treatment, named "soil drying treatment", a decrease of ψ was induced by dehydrating entire plants by withholding water. During this desiccation period, shoots were sampled and ψ_{xyl} was determined at predawn (ψ_{PD}). For the "diurnal transpiration treatment", the decrease of leaf ψ (ψ_{leaf}) was caused by diurnal transpiration of whole, well-watered plants. In the "lab transpiration treatment", ψ of detached shoots was lowered via transpiration under lab conditions. For this, shoots were rehydrated in a 0.01 M KCl solution for 24 h in darkness until ψ_{xyl} of adjacent 3rd order twigs reflected a well-balanced water status. A well-balanced status was attested if ψ at all measured 3rd order twigs mirrored the same level of maximum ψ of −0.1 MPa. Shoots were then dehydrated in the lab for different periods to obtain plant material differing in ψ_{xyl} and K. After two hours of storage in plastic bags, equilibrated shoots were subjected to measurements of K. This treatment was applied to well-watered, potted or field-grown plants.

For all three treatments, twigs of 3rd order on top of 2nd order shoots were excised with a scalpel and ψ_{xyl} of 3rd twigs was measured with a Scholander pressure bomb (SKPM 1440, Skye Instruments Ltd., UK). Directly afterwards, the corresponding 2nd order shoots were used for measurements of K. They were cut from the plant under water and submerged under a 0.01 M KCl solution to avoid air penetrating into xylem vessels.

Hydraulic conductivity

Hydraulic conductivity was determined as the water flow rate (WFR) per gravitation-induced pressure gradient (ΔP) (SPERRY et al. 1988; SCHALLER and PASCHOLD 2008). It was calculated as follows, considering the segment length (l) and the water volume (ΔV) measured per period (t):

$$K = \frac{\Delta V \times l}{\Delta P \times t}\left[\frac{m^3 \times m}{MPa \times s}\right]$$

A segment length of 30 cm was used since the maximum vessel length of 2nd order shoots was 25.7 cm for 'Gijnlim' and 28.2 cm for 'Grolim' (determined using a modified method of ZIMMERMANN and JEJE 1981; SCHALLER 2008). The $K_{initial}$ and K_{max} of single shoot segments are required to quantify xylem cavitation via calculating the drought-induced loss of K (SPERRY et al. 1988; SPERRY and TYREE 1988). The first measurement of K, recorded immediately after inserting the segment into the apparatus (SCHALLER and PASCHOLD 2008), is influenced by native cavitation or embolism in the segment and represents $K_{initial}$, measured with ΔP of 0.01 MPa. To reveal the appropriate hydraulic head for K measurements, the initial water flow rate (WFR) per ΔP was calculated in eight shoot segments ten times consecutively, while applying a ΔP of $2.5*10^{-3}$, $5.0*10^{-3}$, $7.5*10^{-3}$ and 0.01 MPa, respectively, in 2006. Shoots, originating from well-watered plants of 'Gijnlim', were sampled at midday. At this time different levels of ψ_{xyl} occurred in plants. For the appropriate determination of K_{max}, the efficiency of eluting cavitation from xylem vessels was tested by repeating the procedure of flushing and measuring K_{max} (see results, Fig. 1). The flushing procedure with the following parameters – degassed 0.01 M KCl solution, ΔP = 0.08 MPa and t = 30 min – was repeated three times and WFR was measured four times after each flushing.

The K_{max} was determined with ΔP of 0.01 MPa after flushing. Measurements were conducted under laboratory conditions at a temperature of 20 °C. The percentage loss of K (PLC) was computed as:

$$PLC = 100\frac{(K_{max} - K_{initial})}{K_{max}}$$

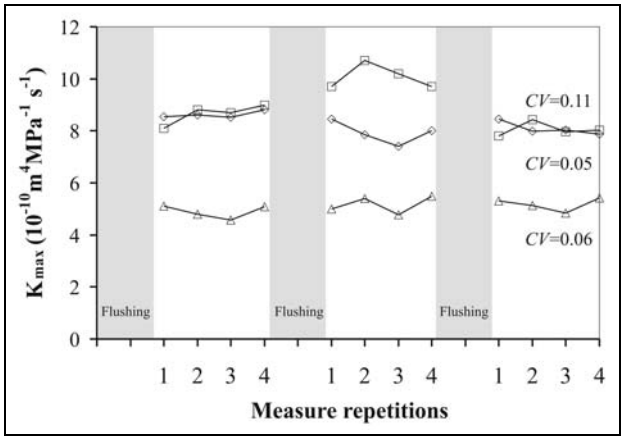


Fig. 1. Maximum hydraulic conductivity (K_{max}) in three shoot segments of *Asparagus officinalis* L. (square, triangle and rhombus) measured at ΔP = 0.01 MPa, after shoots were flushed once, twice and three times with 0.08 MPa, respectively. Low coefficients of variance (CV) of 12 measure replicates prove the flushing procedure as effective in cavitation exclusion.

Vulnerability curves

PLC and ψ_{xyl} were plotted to create vulnerability curves (VC) showing the loss of K with increasing drought stress. Each data point represents a single shoot segment. The curves were calculated by fitting an exponential sigmoidal equation $PLC = 100(1+\exp(-(\psi_{xyl}-\psi_{50})b^{-1}))^{-1}$ with the compiled data. As a result the parameters ψ_{50} and b are calculated (Pammenter and Van der Willigen 1998). The inflexion point ψ_{50} indicates the xylem tension at which a 50 % loss of K occurs and represents the critical level of cavitation. The regression coefficient b is the slope indicating the xylem resistance to cavitation. Small b-values represent low resistance. The estimated PLC at maximum ψ_{xyl} – measured in asparagus (–0.1 MPa) after previous cycles of drought-induced decrease in ψ_{xyl} – indicates the level of native embolism in lateral shoots and is termed PLC_0. Maximum ψ_{xyl} was reached at predawn after water uptake and water balance overnight.

Statistical analysis

Statistical analyses were performed with the open source software R (R Development Core Team 2008). Significances of differences in xylem vulnerability between genotypes in 2006 and months in 2008 were determined performing an analysis of covariance with linearized values of ψ_{xyl} as covariate. PLC-values were tested for normality and homoscedasticity. The regression models and the regression coefficients were considered significant if $P < 0.05$. Level of significance is indicated with * if $P < 0.05$, ** if $P < 0.01$ and *** if $P < 0.001$. Tests of normality and t-tests were applied in case of differences in the means of K and considered significant if $P < 0.05$.

Results

Pressure gradient for K measurements and cavitation exclusion

The coefficients of variance (CV) of repeated $K_{initial}$ measurements varied at $\Delta P = 2.5*10^{-3}$ MPa between 0.29 and 0.58, at $\Delta P = 5.0*10^{-3}$ MPa between 0.43 and 1.01, and at $\Delta P = 7.5*10^{-3}$ MPa between 0.14 and 1.41. A ΔP of 0.01 MPa resulted the lowest CV of 0.21–0.45. These most stable values of $K_{initial}$ indicate the absence of air displacement. This hydraulic head generated higher WFR and ascertained a higher accuracy of measurements with the weighing of exudates. The exclusion of embolism in xylem vessels was realized by applying a $\Delta P = 0.08$ MPa for 30 min (Fig. 1), since repeated flushing procedures did not alter K values.

Shoot treatments and vulnerability curves

With the "soil drying treatment" the exponential sigmoid regression of VC did not fit the measured ratio of PLC and ψ_{PD} ($R^2 = 0.21$, Fig. 2). At ψ_{PD} of –0.3 MPa – corresponding to well-watered potted plants – shoots displayed a wide range of PLC indicating high variation of cavitation level at predawn notwithstanding water supply.

The PLC in shoots attached to transpiring potted plants was measured with the "diurnal transpiration treatment" ($R^2 = 0.69$, Fig. 3). The two genotypes did not differ in their xylem susceptibility to cavitation ($P = 0.573$). The PLC_0 was 8.8 %. The cavitation increase accounted for 0.37 ($P < 0.0001$) and the ψ_{50} for –0.86 MPa ($P < 0.0001$).

With the "lab transpiration treatment" ψ_{xyl}–values were adjusted in small and regular intervals facilitating the creation of VC ($R^2 = 0.60$, Fig. 4). The PLC_0 was 29.0 %. The cavitation increase came to 0.47 ($P < 0.0001$) and the 50 % loss of K emerged at –0.31 MPa ($P = 0.0003$). The two genotypes did not differ in their xylem susceptibility to cavitation in this treatment, too ($P = 0.155$).

Seasonal shift of xylem vulnerability

In 2008, VC were established with shoots of 'Gijnlim' grown in the field (Fig. 5). Individual shoots were subjected to the "lab transpiration treatment" in July (Fig. 5a), August (Fig. 5b) and September (Fig. 5c). The influence of the vegetation period on xylem vulnerability was found to be significant ($P = 0.011$). The generation of cavitation in xylem, including ψ_{50} and b, differed significantly between the months ($P = 0.032$). The curves were differentiated by the critical ψ_{50}, highlighted with vertical bars. The critical cavitation level shifted from –0.34 in July to –0.21 MPa ($P = 0.0001$) in September. The cavitation resistance decreased during the same period with b-values in July of 0.21 ($P < 0.0001$), in August of 0.15 ($P = 0.0002$) and September of 0.11 ($P = 0.0028$). The discrepancy in xylem vulnerability was highest between July and September.

Discussion

The methodical approach for identifying the appropriate hydraulic head for measuring K – particularly for $K_{initial}$ – of this monocot herbaceous species revealed a ΔP of 0.01 MPa. This result is based on the condition of constancy and measuring accuracy in the WFR of shoot segments with a length of 30 cm. Requirements on measurements of K_{max} were met considering exclusion and gener-

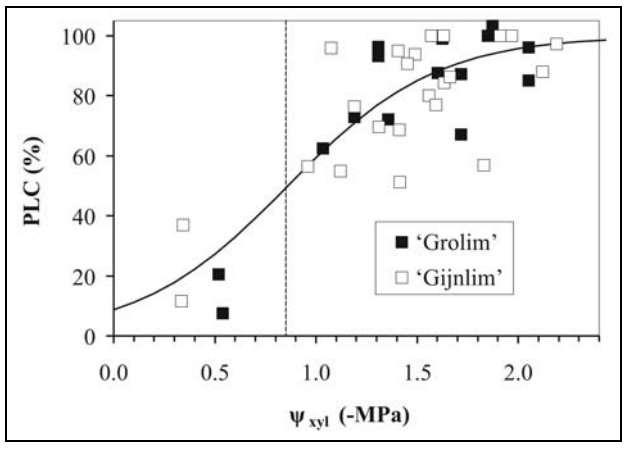


Fig. 2. Vulnerability curve of *Asparagus officinalis* L. 'Gijnlim' and 'Grolim' subjected to the "soil drying treatment" in 2006. The relationship between percentage loss of K (PLC) and xylem water potential (ψ_{xyl}) was calculated with the equation $f(\psi_{xyl}) = 100(1+\exp(-(x-0.70)0.6^{-1}))^{-1}$, $R^2 = 0.21$, n = 57. The vertical bar displays the critical cavitation level of ψ_{xyl}.

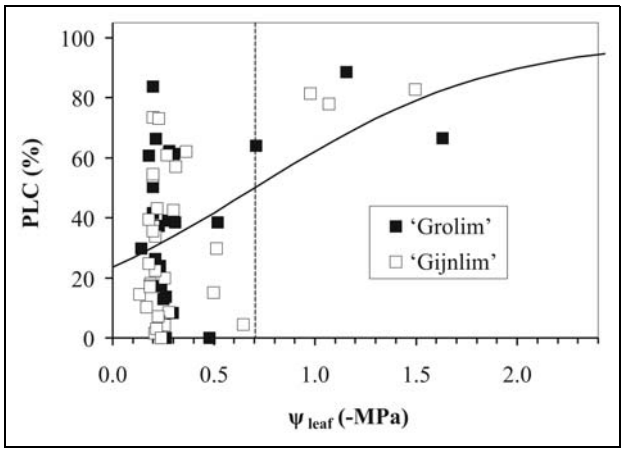


Fig. 3. Vulnerability curve of *Asparagus officinalis* L. 'Gijnlim' and 'Grolim' subjected to the "diurnal transpiration treatment" in 2006. The relationship between percentage loss of K (PLC) and leaf water potential (ψ_{leaf}) was calculated with the equation $f(\psi_{leaf}) = 100(1+\exp(-(x-0.86)0.37^{-1}))^{-1}$, $R^2 = 0.69$***, n = 39. The vertical bar displays the critical cavitation level of ψ_{leaf}.

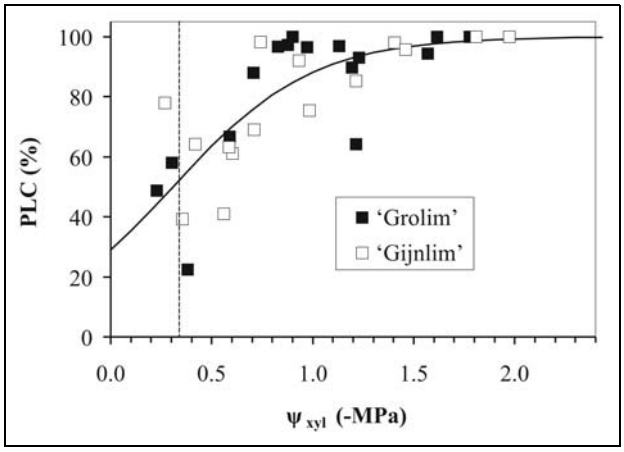


Fig. 4. Vulnerability curve of *Asparagus officinalis* L. 'Gijnlim' and 'Grolim' subjected to the "lab transpiration treatment" in 2006. The relationship between percentage loss of K (PLC) and xylem water potential (ψ_{xyl}) was calculated for 'Gijnlim' with the equation $f(\psi_{xyl}) = 100(1+\exp(-(x-0.31)0.34^{-1}))^{-1}$, $R^2 = 0.60$**, n = 31. The vertical bar displays the critical cavitation level of ψ_{xyl}.

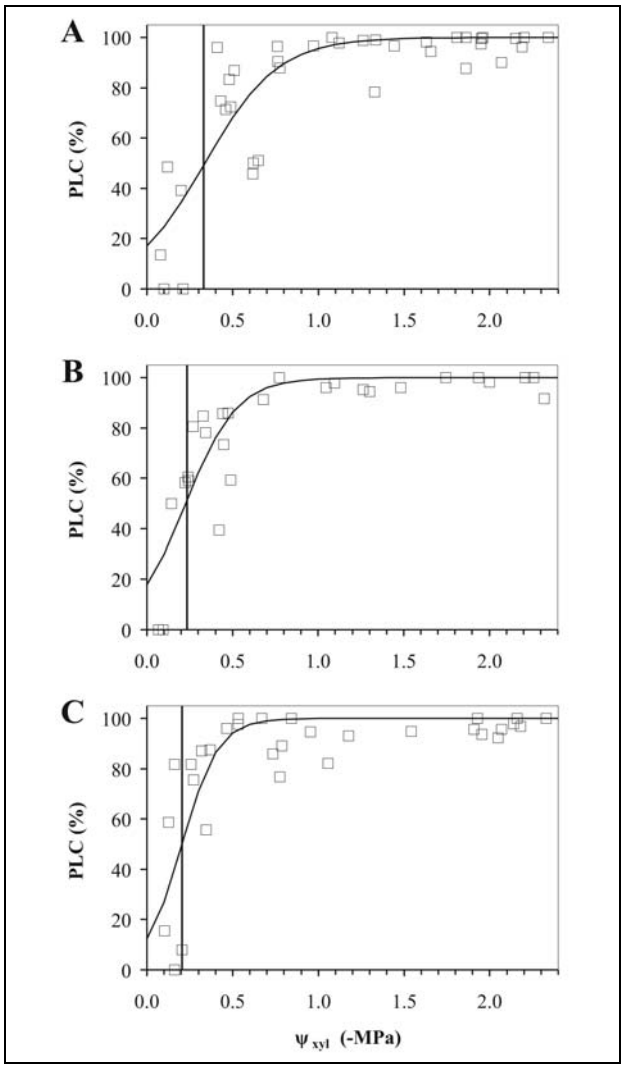


Fig. 5. a–c. Vulnerability curves of *Asparagus officinalis* L. 'Gijnlim' subjected to the "lab transpiration treatment" in July (A), August (B), and September (C) 2008. The relationships between percentage loss of K (PLC) and xylem water potential (ψ_{xyl}) were calculated in (A) with the equation $f(\psi_{xyl}) = 100(1+\exp(-(x-0.34)0.21^{-1}))^{-1}$, $R^2 = 0.72$**, n = 37; in (B) with $f(\psi_{xyl}) = 100(1+\exp(-(x-0.23)0.15^{-1}))^{-1}$, $R^2 = 0.75$**, n = 29; in (C) with $f(\psi_{xyl}) = 100(1+\exp(-(x-0.21)0.11^{-1}))^{-1}$, $R^2 = 0.61$**, n = 31. Vertical bars display the critical cavitation level of ψ_{xyl}. Xylem vulnerability differs between the months (ANCOVA, $P < 0.05$).

ation of cavitation during flushing (Espino and Schenk 2011).

Based on this methodical approach, VC were created for three drought-inducing treatments for releasing xylem embolism in asparagus shoots in order to determine PLC under near-natural conditions. The "soil drying treatment" did not generate a relationship between PLC and ψ_{xyl}. During this treatment, nocturnal refilling of cavities is suggested for well-watered plants unlike drought stressed ones. The highest ψ_{PD} corresponded to a wide range of PLC, indicating incomplete embolism reversal in some shoots overnight (Domec et al. 2006). Root and osmotic pressure in potted plants, involved in the refilling of cavitated vessels (Sperry et al. 2003; Stiller et al. 2003), were probably too weak to reverse PLC. The ψ_{PD} of well-watered plants in pots was more negative than ψ_{xyl} of saturated shoots collected from field-grown plants. Since refilling is a function of internal pressure imbalance, the water potential difference between potted and field plants suggests that the embolized vessels of potted plants were incompletely refilled during the night (Domec et al. 2006). Studies on PLC of well-watered, field-grown asparagus plants at predawn may reveal the impact of root pressure on refilling embolisms in this species. A concluding assessment of this treatment – requiring an investigation under field conditions – is difficult, since the wide range of ψ_{xyl} – is hard to obtain under field conditions.

The "diurnal transpiration treatment" showed the highest coefficient of determination. Still it seems inappropriate for establishing VC, since graduated ψ_{leaf} values in the range between −0.6 and −0.9 MPa were hardly to achieve. In addition, this treatment generated a higher variation of PLC-values at $\psi_{leaf} < -1.2$ MPa. This suggests a daily variation in K, as observed in roots of other non-woody plants (McCully 1999). Diurnally varying cavitation (Hacke and Sperry 2003; Domec et al. 2006) may have caused a hysteresis in the VC and this is supported by higher PLC at a certain ψ-level in the afternoon than in the morning. If hysteresis cannot be excluded, the use of the diurnal transpiration method must be regarded questionable for the determination of VC. Cavitation fatigue – a weakened refilling of embolized vessels after multiple cycles of cavitation and refilling – might explain this hysteresis (Hacke et al. 2001; Stiller and Sperry 2002).

A hysteresis effect can be ruled out in VC created by the "lab transpiration treatment". It is favoured here, because it releases cavitation in a separated hydraulic system of excised fully turgid shoots, avoiding effects of chemical and hydraulic signals from roots or neighbouring shoots on ψ_{xyl} and alleviating effects of short-term stress prehistory. Shoot rehydration was successfully used also for the creation of VC for *Acer saccharum* (Sperry et al. 1988) and *Eucalyptus* hybrids (Pammenter and Van der Willigen 1998) when shoot water status was equilibrated. In our study, rehydration of herbaceous shoots equilibrated water status, but did not reverse embolism completely: 29 % of embolized vessels did not refill. This PLC_0 may suggest cavitation fatigue in this anisohydric species (Schaller 2008), resulting in the risk of daily embolism. However, the high PLC_0 may be due to the lack of root pressure in excised shoots during the night, since PLC_0 in the diurnal transpiration treatment was distinctively lower as revealed with the "lab transpiration treatment". Root pressure has been associated with cavitation reversal in *Oryza sativa* (Stiller et al. 2003). Yet it remains unclear whether the determined level of native embolism in asparagus is real, or is due to a methodical effect of the favoured shoot treatment. Further research on xylem vulnerability of field plants might clarify the specific level of native embolism after nocturnal water status equilibrium, when plants were subjected to several cycles of cavitation and refilling.

The major task of this study was to test the hypothesis that cavitation resistance of asparagus is subject to seasonal changes. A seasonal effect on hydraulic vulnerability to cavitation was detected in plants of 'Gijnlim' under field conditions in 2008. This observation is analogous to seasonal changes of hydraulic vulnerability of shoots and roots of woody plants (Cochard et al. 1994; Kolb and Sperry 1999; Vogt 2001; Maherali et al. 2004; Domec et al. 2006; Jacobsen et al. 2007). Cavitation resistance decreased from July to September. A shift to increased cavitation resistances during the course of the season was found for *Artemisia tridentata* (Kolb and Sperry 1999). In our case, changing resistance may not be explained by water shortage, since the rainfall in July and August 2008 exceeded the long-term mean precipitation (German Weather Service). In addition the crop was irrigated. Considering the absence of water shortage, the seasonal shift to xylem susceptibility may rather be a response to elevated vapour pressure deficit resulting in an increased demand of transpiration in the period before measurement. Elevated vapour pressure deficit is associated with a higher temperature in the preceding months May, June and July in 2008 exceeding the long-time mean (German Weather Service). Considering the effect of a high transpiration demand on PLC, demonstrated in the VC of the "diurnal transpiration treatment" (Fig. 3), repeated diurnal xylem cavitation and nocturnal refilling may promote cavitation fatigue, resulting in an increased xylem vulnerability of vessels of monocot shoots.

Information about seasonal effects on monocot xylem vulnerability is not yet available. Maturation of dicot xylem, changes in mechanical strength and in pectin composition of pit membranes are discussed as the reasons for the seasonal shifts in cavitation vulnerability of woody species (Kolb and Sperry 1999; Gascó et al. 2007; Jacobsen et al. 2007). However, herbaceous monocots are unable to form secondary xylem, and this complicates comparisons with woody species as xylem maturation may not explain the decreased cavitation resistance observed in asparagus. Other possible reasons for the decrease in cavitation resistance in asparagus are aging of primary xylem, associated with shoot age, particularly of vessel elements, and changes in vessel characteristics, which affect hydraulic vulnerability. Perforation plates may become brittle and offer less hydraulic resistance (Zimmermann 1983; Schulte et al. 1989) and possibly less resistance for transmitting cavitation in adjacent vessels of older tissue. Gascó et al. (2007) found that physiological characteristics, such as ionic composition of the xylem sap, alleviated the hydraulic effect of cavitation during the season. Therefore, the lower PLC early in the summer may have been evoked by seasonal hydrogel of the pit membranes that can be induced by modifying the ionic composition of the xylem sap (Gascó et al. 2007).

Conclusion

The shoot treatment of rehydrating and dehydrating excised shoots to obtain corresponding ψ_{xyl} and PLC-values represents the most promising method among the three methods tested for the quantification of cavitation resistance. This "lab transpiration treatment" enabled to identify the xylem vulnerability of the herbaceous monocot species asparagus. Seasonal changes in cavitation resistance occur even without water deficit. This shift of the critical ψ_{xyl} should be taken into consideration, if ψ_{xyl} is used for plant-based irrigation scheduling of asparagus. Research regarding changes in anatomy of monocot xylem during the season may help to explain the unexpected seasonal shift in xylem vulnerability of the primary xylem.

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